Studies on the *Mytilus edulis* Community in Alamitos Bay, California: VII. The Influence of Water-Soluble Petroleum Hydrocarbons on Byssal Thread Formation

BY

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INTRODUCTION

Mytilus edulis, Linnaeus, 1758, is the predominant organism associated with docks, pilings, and boat floats in Alamitos Bay (REISH, 1964a). Occasionally red tide blooms decimate the *M. edulis* population as well as the diverse assemblage of polychaetes, amphipods, and other fauna which comprise the *M. edulis* community (REISH, 1964a, 1964b). It is not known whether the mussels were affected directly by an accumulation of a toxic substance secreted by the dinoflagellates or indirectly by the decrease of dissolved oxygen in the water due to bacterial decomposition of organic material. A drastic reduction in byssal thread production was observed under laboratory conditions for *M. edulis* exposed to dissolved oxygen concentrations below 0.9 ppm (REISH & AYERS, 1968), which is higher than the 0.1 ppm observed in Alamitos Bay during the 1962 red tide.

It seems logical to assume that byssal thread production is a reasonable parameter with which to gauge the metabolic activity or physiological functioning of *Mytilus edulis*. The ability of *M. edulis* to form byssus attachments is crucial to the survival of this organism. MARTIN *et al.*, (1975) have shown that byssal thread production may be used as an indicator of the dose response to heavy metal toxicants.

Due to the activity of motor powered vessels within the Alamitos Bay area, petroleum hydrocarbons are a pollutant with which the *Mytilus edulis* community must contend. The purpose of this present investigation was to determine what effect the water-soluble fraction of three different oils had on the capacity of *M. edulis* to form byssal threads in the laboratory.

MATERIALS AND METHOD

Mytilus edulis specimens were collected from boat docks in Alamitos Bay during the month of July. Only mussels from 18 to 23 mm in width were used. Limiting specimens to this size range insured that no gravid individuals would be included. GLAUS (1968) showed that small *M. edulis* produce more byssal threads than do larger ones under optimum conditions. Several hundred mussels of the appropriate size were acclimated at $19.5 \pm 0.5°$ C for three days. Prior to the start of the experiment, the valves were scraped clean of fouling organisms and all byssal threads were cut.

Two of the oils used in this study were South Louisiana crude oil and No. 2 fuel oil which were originally supplied by the American Petroleum Institute and redistributed by Dr. Jack W. Anderson of Texas A&M University. A commercially available oil, Sta-lube 50:1 2 cycle outboard motor oil was also tested. The procedure for preparation of the water-soluble fraction (WSF) of the oils was similar to the method employed by ANDERSON *et al.*, (1974a). Nine parts millipore filtered (0.45 μm) seawater was stirred with one part oil on a magnetic stirrer for 20 hours at a slow speed. After stirring, the aqueous phase was siphoned off and utilized immediately in experiments. A detailed liquid-gas chromatographic analysis of the two API reference oils and their WSFs was carried out earlier by Dr. J. Scott

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Warner, Battelle Memorial Laboratories (ANDERSON *et al.*, 1974).

All experiments were conducted at 19.5 ± 0.5° C with millipore filtered (0.45 μm) seawater. The bioassay containers employed were 500 mL Erlenmeyer flasks. Each flask contained 100 mL of the appropriate test solution with one mussel per flask. Concentrations of 25, 50, 75, and 100% of the initial WSF were tested for each of the three oils. The solutions were not changed or replenished during the course of the experiment. No food was administered throughout the duration of the experiment. Byssal thread production and survival were observed at 1, 2, 3, 4, 7, and 14 days. Because the data did not conform to analysis by parametric techniques, the non-parametric ranking test of Mann and Whitney was utilized to determine significant differences in byssal thread production.

RESULTS

The influence of the WSFs of South Louisiana crude oil, No. 2 fuel oil and Sta-lube outboard motor oil are presented in Tables 1, 2, and 3, respectively. Byssal thread production was significantly inhibited at the 100% WSF for all three oils. For South Louisiana crude oil and No. 2 fuel oil this inhibitory influence was significant throughout the duration of the experiment. For the Sta-lube outboard motor oil the inhibition of byssal thread production was only significant up until the 72 hour check. South Louisiana crude oil was most effective in its inhibitory influence on a percent WSF basis while the Sta-lube outboard motor oil produced the least effect.

An unexpected result of this experiment was that a significant enhancement in byssal thread production was observed for all three WSFs at concentrations below those at which an inhibitory effect was elicited. At 96 hours a significant enhancement was observed at 25% of the WSF for South Louisiana crude and No. 2 fuel oil while 50% of the WSF of Sta-lube outboard motor oil produced a similar stimulation as compared with the control. In a repeat experiment, 25% WSF of No. 2 fuel oil produced a fourfold increase in byssal thread production as compared with the control after 96 hours of exposure. This stimulation of byssal thread formation, however, was not significant beyond 96 hours for any of the three WSFs.

DISCUSSION

The WSF of South Louisiana crude oil and No. 2 fuel oil have been analyzed by UV and IR spectrophotometry and were shown to contain 19.8 mg/L and 8.7 mg/L total hydrocarbons, respectively (ANDERSON *et al.*, 1974a). Of the hydrocarbons in the No. 2 fuel oil WSF, approximately 39% are aromatic compounds as compared to less than 2% aromatics for the WSF of South Louisiana crude oil. The soluble aromatic and naphthalene compounds of an oil produce the majority of its toxic effect (ANDERSON *et al.*, 1974b). These compounds are highly volatile, however, as the naphthalene compounds have been observed to reach undetectable levels under similar bioassay conditions after 96 hours (CARR & REISH, 1977). The higher molecular weight ($> C_{10}$) water-soluble paraffinic hydrocarbons are not nearly as volatile as the aromatic ones (DODD, 1974), and hence their relative percentage of the total hydrocarbons present in solution increases with time.

An examination of Tables 1 and 2 indicates that South Louisiana crude oil was the most effective inhibitor of byssal thread production on a percent WSF basis. Since the WSF of South Louisiana crude oil contains the highest concentration of paraffinic hydrocarbons, which are the least volatile of the water-soluble hydrocarbons, it appears likely that these higher molecular weight ($> C_{10}$) aliphatic compounds are at least partially responsible for the chronic inhibition of byssal thread formation. In the two refined oils, whose WSFs contain a higher percentage of volatile aromatics, the suppression of byssal thread formation was not nearly as marked.

Other studies have shown the WSF of No. 2 fuel oil to be more toxic than South Louisiana crude oil on a percent WSF and total initial hydrocarbon basis for a variety of marine organisms (ANDERSON *et al.*, 1974; ROSSI *et al.*, 1976; CARR & REISH, 1977). In the present study at 14 days the 100% WSF crude oil group suffered 40% mortality whereas in the 100% WSF of the No. 2 fuel oil only 20% mortality was observed. These were the only deaths occurring during the course of the experiment. While this difference in survival rates is not statistically significant, the relative mortality rate follows the trend seen in suppression of byssal thread production. Again, the most deleterious effects were produced by the WSF of South Louisiana crude oil. It appears that unlike most of the other marine organisms which have been tested that *Mytilus edulis* is more susceptible to contamination by crude oil than by these refined oils.

The unanticipated result of a statistically significant stimulation by byssal thread production was observed for all three WSFs up until 96 hours after which time the stimulatory effect although evident was not significant statistically. When considering the changes in hydrocarbon composition occurring in the WSF over a 96 hour time

Table 1

Influence of Seawater-Soluble Fraction (WSF) of South Louisiana Crude Oil on Byssal Thread Production of *Mytilus edulis.* Tests for Statistical Observations and Significance.

	24 hours		48 hours		72 hours		96 hours		7 days		14 days	
Experimental Concentrations as Percent WSF	Average No. of Byssal Threads	U[1]	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U
Control n = 20	5.75	—	9.85	—	12.6	—	15.55	—	28.4	—	39.15	—
25 n = 10	8.2	123.5	17.3	150[2]	18.6	133	24.6	146.5[2]	42.3	130	58.6	131.5
50 n = 10	2.5	137	5.1	126.5	5.7	146[3]	8.2	133.5	12.0	131.5	21.4	125.5
75 n = 10	1.1	159.5[3]	3.2	151.5[3]	3.4	1.54[3]	3.9	171[3]	7.5	163.5[3]	15.2	150.5[3]
100 n = 0	0	185[3]	0.1	193[3]	0.1	193[3]	0.1	199[3]	1.4	191[3]	4.1	177.5[3]

[1]Mann-Whitney U statistic comparing experimental populations with the control. U statistic at .05% level of significance = 138.
[2]Significant increase in byssal thread production.
[3]Significant decrease in byssal thread production.

Table 2

Influence of Seawater-Soluble Fraction (WSF) of No. 2 Fuel Oil on Byssal Thread Production of *Mytilus edulis.* Tests for Statistical Observations and Significance.

	24 hours		48 hours		72 hours		96 hours		7 days		14 days	
Experimental Concentrations as Percent WSF	Average No. of Byssal Threads	U[4]	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U
Control n = 20	5.75	—	9.85	—	12.6	—	15.55	—	28.4	—	39.15	—
25 n = 10	13.3	132.5	24.8	146.5[5]	29.4	144[5]	43.2	144[5]	47.2	133.5	57.3	134
50 n = 10	3.3	131.5	9.0	102	13.4	106.5	18.5	123.5	31.3	108	37.2	120
75 n = 10	0.3	178.5[6]	8.8	106.5	11.4	102.5	15.8	102.5	25.3	100.5	39.1	101.5
100	0	185[6]	0.1	197[6]	0.2	190.5[6]	2.8	178[6]	6.8	161.5[6]	16.1	148[6]

[4]Mann-Whitney U statistic comparing experimental populations with the control. U statistic at .05% level of significance = 138.
[5]Significant increase in byssal thread production.
[6]Significant decrease in byssal thread production.

period. the evaporation of the volatile aromatics and naphthalenic compounds. particularly, is the most predominate change. The stimulation of byssal thread formation decreases at approximately the same time that the naphthalenic compounds reach undetectable levels in the WSF, lending credence to the possibility that these aromatic and polyaromatic compounds may be responsible for this observed stimulatory effect. Additional tests with specific aromatic and naphthalenic compounds would further elucidate this hypothesis. Most likely there is no specific hydrocarbon that is causing this effect but rather a combination of compounds which is responsible.

This is not the first time that exposure to low-level water-soluble hydrocarbons has produced a stimulation of some physiological process in marine organisms. NEFF *et al.*, (1976) observed an increased growth rate with zoea of the mud crab *Rhithropanopeus harrisii* exposed to sublethal concentrations of No. 2 fuel oil. TATEM (1975) observed an increase in the number of larvae produced in the grass shrimp *Palaemonetes pugio* when exposed to sublethal WSFs of No. 2 fuel oil. ROSSI (1976), conducting an investigation through several generations with the polychaete *Neanthes arenaceodentata,* observed a decrease in oocyte maturation as compared with the control for all exposure concentrations. Reproduction was significantly stimulated in the polychaete *Ophrytrocha diadema* exposed to an initial total hydrocarbon concentration of 1.99 ppm of South Louisiana crude oil (CARR & REISH, 1977). Similar phenomena have been observed in toxicity tests with rats and mice (SMYTH, 1967). Smyth suggests that the increased growth and reduced mortality observed in toxicity tests with low levels of known noxious substances is due to the exercising of homeostatic mechanisms. It seems likely that a similar phenomenon is occurring in some marine animals exposed to water-soluble hydrocarbons.

When considering the results of these experiments and how they relate to the natural environment, it is worthy of noting that the type of water-soluble hydrocarbons most commonly entering the harbor waters (outboard motor fuel) produces the least inhibition of byssal thread production. Since *M. edulis* characteristically inhabits floating boat docks near the air-water interface, these mussels would be exposed to a greater threat than that posed by water-soluble hydrocarbons were a large oil spill to take place

Table 3

Influence of Seawater-Soluble Fraction (WSF) of Sta-lube Outboard Motor Oil on Byssal Thread Production of *Mytilus edulis.* Tests for Statistical Observations and Significance.

	24 hours		48 hours		72 hours		96 hours		7 days		14 days	
Experimental Concentrations as Percent WSF	Average No. of Byssal Threads	U[7]	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U	Average No. of Byssal Threads	U
Control n = 20	5.75	—	9.85	—	12.6	—	15.55	—	28.4	—	39.15	—
25 n = 10	10.0	132	14.8	122.5	16.6	120.5	20.8	116.5	26.5	104	28.8	106
50 n = 10	16.3	142[8]	21.4	140[8]	25.9	142[8]	31.8	138[8]	39.1	125	48.8	118.5
75 n = 10	2.8	123	8.7	113	11.6	115	18.1	101.5	23.2	104.5	28.1	113
100	1.0	171[9]	4.2	153[9]	4.5	154[9]	10.2	135	22.2	104	27.4	109.5

[7]Mann-Whitney U statistic comparing experimental populations with the control. U statistic at .05% level of significance = 138.
[8]Significant increase in byssal thread production.
[9]Significant decrease in byssal thread production.

locally. Their proximity to the air-water interface would allow floating oil to strand on these mussels causing physical smothering under extreme conditions. Although not as dramatic, chronic exposure to sublethal water-soluble hydrocarbons might also lead to the decline or eradication of a population in a less conspicuous manner.

The results of this study suggest that the levels of water-soluble hydrocarbons currently found in Long Beach Harbor due to motor boat activity pose little threat to the survival of *Mytilus edulis*. The levels of water-soluble hydrocarbons necessary to cause deleterious effects would only be found associated with a local spillage of oil. Certain areas receiving chronic low level hydrocarbon insult may not be suitable for survival of *M. edulis*. Barring the event of any local oil spills, *M. edulis* population levels are more dependent on the periodic red tide phenomenon than the effects resulting from local motor boat usage. Why low water-soluble hydrocarbon levels produce stimulation of byssal thread production is a question which awaits further investigation.

SUMMARY

1. The bay mussel *Mytilus edulis* was exposed to varying concentrations of the water-soluble fraction (WSF) of No. 2 fuel oil, South Louisiana crude oil and Sta-lube outboard motor oil and the number of byssal threads produced was recorded at intervals over a 14 day period.

2. Byssal thread production was significantly inhibited at the 100% WSF for all three oils.

3. South Louisiana crude oil was the most effective in its inhibitory influence on a percent WSF basis while the Sta-lube outboard motor oil produced the least effect.

4. An unexpected result of this experiment was that a significant enhancement in byssal thread formation was observed for all three WSFs at concentrations below those at which an inhibitory effect was elicited.

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